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Bioavailability and Urinary Excretion of Phenolic-Derived Metabolites after Acute Consumption of Purple Majesty Potato in Humans

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Abstract

A novel purple potato variety, Purple Majesty (PM) contains an abundance of phenolic compounds, especially anthocyanins. The aim of this study was to assess the bioavailability of phenolic compounds in plasma measured as total polyphenols and urinary excretion of phenolic-derived metabolites after acute consumption of cooked PM. Five healthy male subjects (27-60 years; mean BMI: 26.7 ± 4.1) participated in a bioavailability study. Blood and urine were sampled at baseline and following consumption of 400 g cooked PM at 1h, 2h, 4h and 24h. A peak plasma antioxidant capacity was reached 1-2 hours post-consumption (from 1044 ± 281 µmol/L Fe(II) at baseline and increased to 1257 ± 180 after 1 hour ($p = 0.045$) and 1112 ± 251 µmol/L Fe(II) after 2 hours (borderline significance of $p = 0.06$). Total phenols level in plasma was reached after 2 hours (from 342.4 ± 28.3 at baseline to 368.4 ± 25 mg/L GAE). Liquid chromatography mass spectrometric (LC-MS) analysis was used to track the levels of anthocyanin-like derivatives and metabolites in the urine of volunteers after intake of the cooked Purple Majesty potatoes. No anthocyanin derivatives were detected in urine by liquid chromatography mass spectrometry indicating levels were < 2 nM. The majority of peaks that increased after intake were putatively identified as sulphated phenolic metabolites. Phenolic glucuronides were identified but other peaks remain unidentified. Hippuric acid was identified as a major phenolic derivative. Hydroxy benzoic derivatives, characteristic of intake of anthocyanins, were not detected in urine, however metabolites expected from the B-ring of petunidin (i.e. methyl gallic acid) may have been obscured by other peaks. Some metabolites could have arisen through metabolism of chlorogenic acid, which is present at ~ equivalent amounts to anthocyanins in cooked PM. In conclusion, acute consumption of PM resulted in an increase in excretion of urinary phenolic-derived metabolites. Identifying these unknown phenolic derivatives warrants further investigation.

Keywords: *Bioavailability; Purple Majesty potato; Urinary excretion; Anthocyanins*

Abbreviations: BMI: Body Mass Index; CVD: Cardiovascular Disease; FRAP: Ferric-Reducing Antioxidant Power; GAE: Gallic Acid Equivalent; LC-MS: Liquid Chromatography Mass Spectrometry; PM: Purple Majesty potato; UPW: Ultra Pure Water; SPE: Solid Phase Extraction; ROS: Reactive Oxygen Species

Introduction

Potato (*Solanum tuberosum L*) is a highly produced crop and is the third largest food crop consumed globally [1]. Potatoes provide a rich and varied source of vitamins, minerals and other phytochemicals in particular, polyphenolic compounds. Polyphenolic compounds are considered to promote beneficial effects on coronary heart disease (CHD) and atherosclerosis [2]. However their biological activity in vivo depends on their bioavailability. Potato is usually not associated with a high antioxidant activity, however due to its high consumption it is thought to contribute to the overall high total phenolic content of the human diet [3]. Purple Majesty (PM) is a naturally occurring variation which has been introduced to Scotland. PM contains anthocyanins (red pigments), in addition to chlorogenic acid, ferulic and caffeic acid. Chlorogenic acid accounts for ca. 90% of the total phenolic content in potato tubers [4]. Several studies have reported beneficial effects following potato consumption including potent antioxidant, anti-microbial and anti-obesity capacities [5]. Moreover, clinical studies have reported a lowering of blood pressure in hypertensive humans [6] and reduced cardiovascular complications in hypertensive animal model [7].

There is limited information on the bioavailability and pharmacokinetics of potato phenolics following human ingestion. However, it is widely accepted that polyphenols undergo substantial metabolism following ingestion. Clifford reported that only 5% of dietary polyphenols are absorbed from the duodenum, and over 95% are fermented by colonic bacteria [8]. A fraction of these microbial metabolites are absorbed and presented in the plasma as conjugates. The absorption rate for dietary polyphenolics varies widely. Ferulic acid was reported to increase rapidly in the plasma of rats reaching a peak urinary excretion at 7-8h, following the consumption of tomatoes [9]. The bioavailability of anthocyanins is poor with reports of 1.5-5% recovered in the urine following wine consumption, and 1.5-1.8% of pelargonidin metabolites found in the urine following ingestion of strawberry puree [10]. Absorption of the anthocyanins appears to be related to the amount ingested diet, the chemical structure and nature of conjugation.

The aim of the present study was to elucidate the bioavailability of polyphenolic compounds in human participants following an acute ingestion of Purple Majesty potato (PM), in addition to their influence on total in vivo antioxidant capacity.

Materials and Methods

Study Design

A study was conducted in five healthy male participants (Age range: 27-60 years) in order to assess the absorption and bioavailability of phenolics derived from Purple Majesty (PM) potatoes following an acute consumption. The study was conducted at Queen Margaret University, Edinburgh, UK, and ethical approval was granted by Queen Margaret University Research Ethics Committee. Participants provided written informed consent and attended a clinical facility at Queen Margaret University, Edinburgh, UK following an overnight fast. Participants were asked to refrain from consuming polyphenol-rich foods (E.g. tea, coffee, fruit juices, red wine, chocolate, apples, broccoli and onions) 72 hours prior to the study. Furthermore, they were instructed to refrain from drinking alcohol-containing beverages and eating fish. Compliance with the dietary restrictions was monitored with a 3-day food diary record.

Sample Collection & analysis

Blood was drawn by venepuncture from the antecubital vein in tubes containing lithium heparin and EDTA as anticoagulant. Blood was sampled at baseline and at 1, 2, 4 and 24h post-consumption of PM. The blood samples were stored on ice no longer than 35 min. Subsequently, plasma was separated by centrifugation (1500g, 10 min, 4°C) aliquoted in three 1.5 mL sample tubes, and stored at -80°C. Urine samples were collected by the participants at 0-2; 2-6 and 6-24h post-consumption of PM. Twenty mL aliquots were taken and stored at -80°C. Antioxidant capacity was determined in the FRAP-derived antioxidant assay as described by Benzie and Strain [11] and total phenolics were assessed in plasma and urine as described previously [12] according to the method of Singleton and Rossi [13].

Solid phase extraction (SPE) of urine samples

Briefly, urine samples (10 mL) were acidified with 100 µL of 50% formic acid in ultra pure water (UPW) and ascorbic acid was added (500 µL of 10 mM ascorbic acid in UPW) as an antioxidant to stabilize the phenolic components. One hundred µL of internal standard was added (1 mg/mL: Morin containing 1 mg/mL cyanidin-3-O-glucoside), vortex mixed well and placed on ice. SPE units (Strat C18E GIGA units, 1000 mg sorbent, 6 mL capacity) were prepared by washing in 80% acetonitrile in UPW then equilibrated in ultra pure water containing 0.5% formic acid using a vacuum manifold. The samples were applied to the individual labelled SPE units in tow 6 ml batches and the unbound samples collected in 15 mL tube. The water wash (3 x 5 mL UPW containing 0.5% formic acid) was performed to ensure all salts were removed. The bound material including internal standards was obtained by eluting with 2 x 5 mL 80% acetonitrile. Ascorbic acid was largely recovered in the unbound samples. The bound samples were then stored for 1h at -80°C then dried on a Speed-Vacuum. Samples were reduced from 10 mL to 1 mL within 2h, frozen then freeze-dried to remove the remaining water. The freeze-dried material was re-suspended in 250 µL of 5% acetonitrile in UPW containing 0.1% formic acid by vortexing. Samples were then centrifuged at 16,000 g for 10 min at 4°C prior to placing in LCMS vials for analysis. LC-MS conditions were as described in the section below.

LC-MS-Based Profiling of Phenolics in Urine

Urine samples were defrosted, mixed by vortex then made up to 10 ng/mL with a stock solution of internal standard, morin (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one) and prepared in filter vials (Whatman Mini-Unipreps, 0.45 µM) for LC-MS. Samples (20 µL injections) were analyzed in triplicate on a LCQ-DECA LC-MS system (Thermo Finnigan Ltd), comprising a Surveyor auto sampler, pump and photodiode array (PAD) detector and a Thermo mass spectrometer ion trap. The PAD scanned three discrete channels at 280, 365 and 520 nm. The LCQ-DECA was fitted with an electrospray ionization (ESI) interface and was used with full scan (80-2000 m/z) in negative mode. The MS was tuned against morin (negative mode) and cyandin-3-O-glucoside (positive mode). The reverse phase separation used a Synergi 4µ Hydro C18 (150 x 2 mm, 4 µm) column (Phenomenex Ltd) with a linear gradient from 5% B (0.1% formic acid in acetonitrile) in A (0.1% aqueous formic acid) to 40% B over 25 min, then to 100% B at 30 min. The flow rate was 0.2 mL min-1. Peaks that increased after juice consumption were identified by comparison of the PDA traces using full scan mode and also the different UV channels using the Xcalibur software.

Statistics

Data are presented as mean values ± standard deviation (SD), n = 5. Each sample was analyzed in triplicate (unless otherwise stated) and calibrated against relevant standards where appropriate. Independent 2-tail paired t-tests were used to assess differences between groups following the input of all data in the SPSS version 19 (Chicago, IL, USA) and used to assess differences between time points. Values at $P < 0.05$ were considered to be statistically significant.

Results and Discussion

A peak plasma antioxidant capacity was reached 1-2 hours post-consumption of PM. Levels ranged from 1044 ± 281umol/L Fe(II) at baseline and increased to 1257 ± 180 after 1 hour ($p = 0.045$) and 1112 ± 251umol/L Fe(II) after 2 hours (borderline significance of $p = 0.06$) respectively. The levels remained higher than baseline up to 24 h post-consumption (Figure 1). In addition, a peak plasma total phenolic concentration was reached after 2 hours post-consumption of PM. Levels ranged from 342 ± 46 mg L^{-1} GAE at baseline and increased to 368 ± 42 mg L^{-1} GAE after 2 hours, but the increase was statistically not significant (Figure 2). The urinary profile showed a progressive increase in antioxidant capacity and total phenolic levels up to 24 hours indicating a prolonged effect following the consumption of purple Majesty.

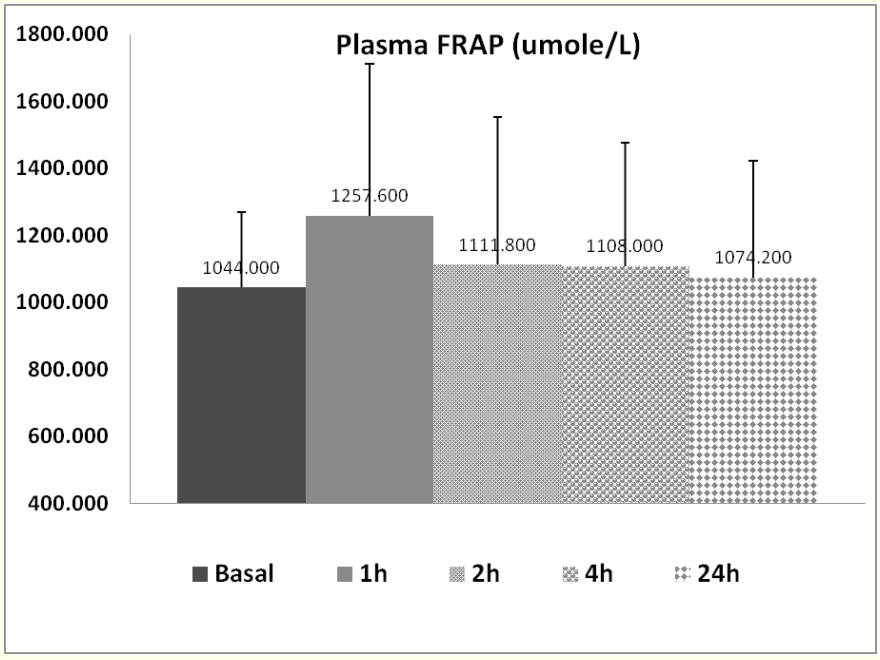

Figure 1: *Antioxidant Capacity of Plasma from baseline-24h post-consumption of Purple Majesty. Results are expressed as mean umol/L Fe^{2+} (n = 5), ± SD. After 1 hour, p = 0.045 and after 2 hours, p = 0.06.*

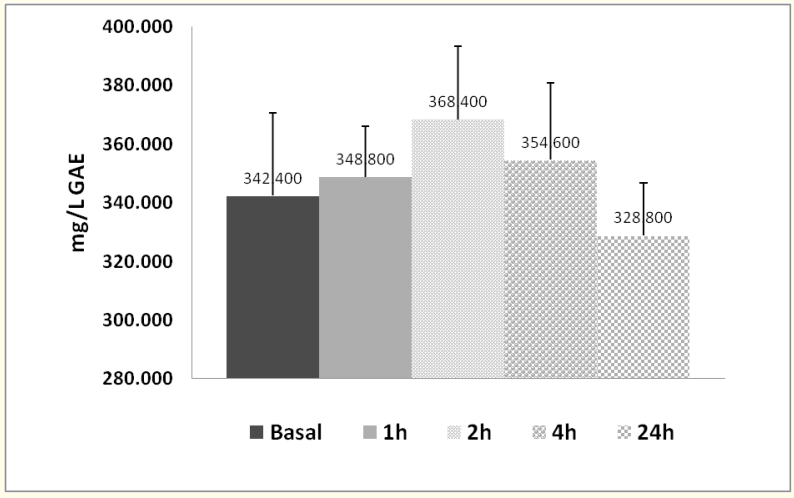

Figure 2: *Total Phenolic concentration of Plasma from baseline-24h post-consumption of Purple Majesty. Results are expressed as mean mg/L GAE (gallic acid equivalent) (n = 5), ± SD.*

Urine samples were subjected to SPE to concentrate the anthocyanin metabolites to facilitate detection by liquid chromatography mass spectrometric techniques. This caused an effective x 40 concentration of the samples with a recovery of internal phenolic standard compounds, including the anthoycanin, cyanidin-3-O glucoside that was greater than 85%. However, no anthocyanin derivatives could be detected in the SPE samples by searching for components that absorb at 520 nm or by searching the mass spectral data for masses characteristic of petunidin derivatives or methylated/glucoronidated/suplphated varients. The limit of detection for anthocyanins on the MS was defined using various standard components at ~ 1 ng/injection. This means that if anthocyanin derivatives are present, they must be present at < 50 µg/L which is equivalent to 0.111 µM or 111 nM (assuming molecular weight of 448 = cyanidin-3-O glucoside) in the SPE concentrate. Allowing for recovery from SPE calculated at a minimum of 75%, this equates to 111/40 x 0.75nM = ~ 2.0 nM. This is in the range of published values from berry intake [14-19] and other sources [20, 23]. However, many of these papers had taken large amounts of berries (several hundred grams) which give substantially more anthocyanin intake than calculated from total anthocyanin intake of the cooked PM (which was estimated at 26 mg anthocyanins/400 g intake).

A range of phenolic metabolites were detected in the urine by LC-MS and some of these increased in abundance after intake of PM. In one subject, the levels of these components appeared to increase within 0-2h. However, in most subjects, the same peaks were only noted as increasing if the traces were compared against the 0-2h urine samples rather than the baseline urine samples (Figure 3). This may be the result of the highly concentrated overnight urine samples or may reflect non-adherence to the low phenolic intake recommended before the trial.

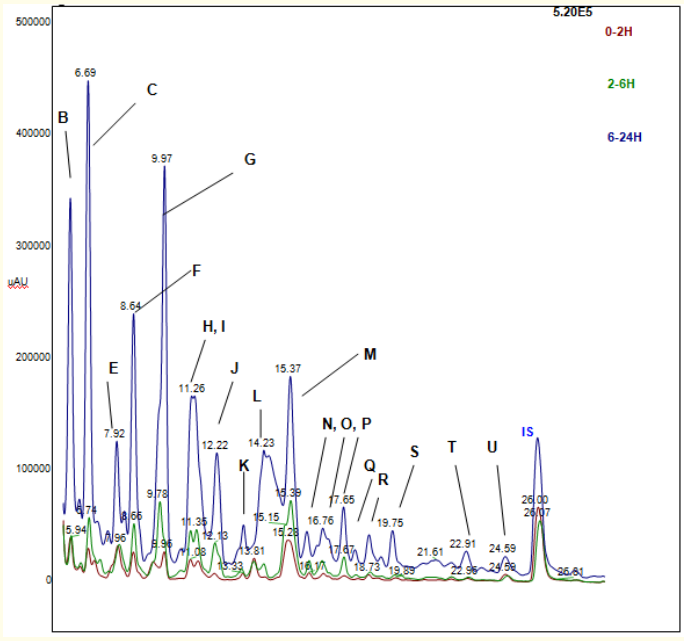

Figure 3: *Peaks present in urine sample from subject 3 with baseline removed Table 1.*

The differences in the pattern of increase of certain peaks is due to inter-individual difference in metabolic efficiency, the volume of urine excreted and, particularly in 6-24h samples, differences in bacterial populations present in the colon. Many of the peaks contained phenolic metabolites recognizable by their PDA and MS properties. However, some peaks remain unidentified. The presence of many of these metabolites (ferulic, caffeic acid and their derivatives, dimethoxycinnamic acid, dehydroxyphenylacteic acid etc) is consistent with current theories on metabolism of anthocyanins in the body [15,19,20].

Schematic 1 - Degradation of petunidin at the C-ring to produce products

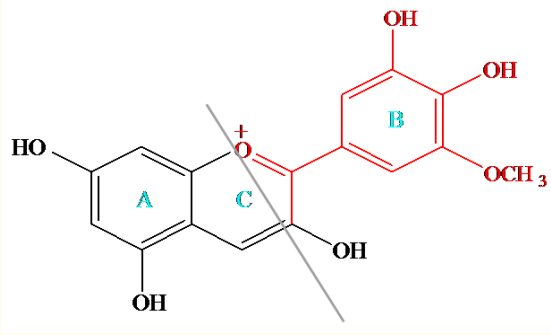

Figure 4: *Schematic diagram showing degradation of petunidin at the C-ring to produce products.*

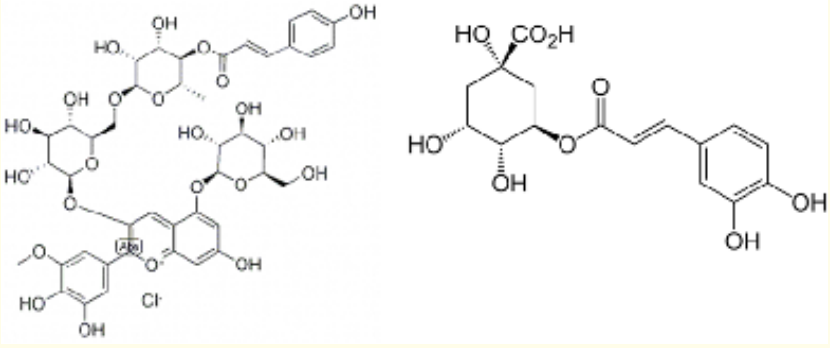

Figure 5: *Schematic diagram showing the structure of petanin and chlorogenic acid.*

The majority of increased peaks were putatively identified from their PDA and mass spectral properties (Table 1) as sulphated phenolic metabolites. The presence of such phenolic derivatives in urine has been noted previously [17,21] and they are characterized by their loss of 80 amu upon fragmentation. Other putative phenolic metabolites, including glucuronides, were identified. Hippuric acid was identified as a major phenolic derivative and this agrees with the findings of Hollands et al. [14] who identified this component and its hydroxylated derivatives as a major urinary phenolic product after anthocyanin intake from black currants.

Peak	RT (min)	PDA	m/z [M-H]	MS^2	Putative ID
A	4.62	255	323.93	236.89, 174.78, 148.01	Phenolic-GlcA (148-GlcA)
B1	5.41	281	181.07	181.07, 137.18	Homovanillic acid /dihydrocaffeic acid
B2	5.97	271	218.98	138.91, 80.98, 79.98	139-S
C1	6.71	265	365.01	244.91, 208.91	UK
C2	7.17	257	227.97	227.92, 148.06, 146.01, 121.09, 79.95	148-S
D	7.48	257	246.86	246.83, 166.98, 123.30	167-S (diOHPA-S)
E	7.95	254	195.07	195.07, 99.99, 93.05	Dihydroferulic acid
E2	8.88	271	218.96	218.91, 154.87, 138.90, 79.93	139-S
F	9.81	253	873.09	873.09, 582.13	UK
G	9.99	284	195.07	195.07, 99.96, 93.07	Dihydroferuli acid[A]
H	11.12	267	304.98	206.83, 163.01	diMeCA derivative?
I	11.30	286	193.02	150.03	(iso)ferulic acid[B]
J	12.15	266	382.92	320.87,206.99,174.74,112.91	207-GlcA (dimethoxy CA)
K	13.69	279,320	260.96	180.94,135.00,80.13	Dihydrocaffeic-S
L	14.37-17.73	278	212.00	211.94,132.03,81.00,80.00	132-S (indoxyl-S)[C]
M	15.36	238	177.90	177.91,133.99,159.70	Hippuric acid
N	16.04	295	272.89 262.95	192.9 144.96, 126.94, 108.70	193-S (ferulic acid) UK
O	16.82	316,295	316.96	none	UK
P	17.65	270	295.06	none	UK
Q	18.73	343,293 279	186.95	186.95, 107.05,80.94,79.96	CRESOL-S
R	19.42	279	372.07	292.17	292-S
S	19.79	276, 323	495.06	319.06	319-GlcA
T	22.90	273	389.00	388.93,306.98,262.9	UK
U	24.59	273	539.23	none	UK

Table 1: *Peaks identified containing phenolic metabolites in the urine of participant 1.*

A: hydroxyhippuric acid also has m/z [M-H] = 193.

B: m/z [M-H] = 195 could also be due to dimethoxyphenylacetic acid.

C: The presence of indoxyl sulphate is speculative and requires confirmation.

S: Sulphate group (loss of 80 amu); GlcA: Glucoronide group (loss of 176 amu); DiMeCA: dimethoxycinnamic acid; diOHPA: dihydroxyphenyl acetic acid.

The increased levels of (iso) ferulic and caffeic acid sulphate derivatives have been noted previously after anthocyanin intake [15]. Degradation at the C-ring can produce cinnamic acid derivatives from the A-ring which undergo subsequent methylation and sulphation and this could be the course of the putative dimethoxycinnamic acid derivatives. Further metabolism by bacteria can produce phenylacetic and phenylpropionic derivatives.

This study did not identify an increase in urinary levels of hydroxybenzoic derivatives which are also characteristic of intake of anthocyanins [15] even by searching the MS data at specific ion masses. These metabolites results from the B-ring of the anthocyanin core after C-ring fission (Figure 4) [18,20], which can occur spontaneously at physiological pH of the small intestine or serum. However, it is possible that benzoic acid metabolites expected from the B-ring of petunidin (such as methyl gallic and syringic acid, after methylation) have been obscured by other peaks. However, it is notable that most of the metabolites identified so far could have arisen through metabolism of the caffeic acid moiety of chlorogenic acid, which is present in roughly equivalent amounts as petanin in cooked PM (Figure 5) [24].

In summary, acute consumption of 400g of Purple Majesty showed a marked increase in the levels of total phenolics in the plasma and urine with enhanced antioxidant capacity in healthy volunteers. The results suggest that phenolic compounds from Purple Majesty are absorbed into the bloodstream and are bioavailable as evidenced by the peak plasma antioxidant capacity 1-2 hours post-consumption. The urinary profile shows a progressive increase in antioxidant capacity and total phenolics levels up to 24 hours indicating the prolonged effect following the consumption of purple Majesty [25].

Conclusions

Acute consumption of Purple Majesty potato resulted in an increase in excretion of urinary phenolic-derived metabolites. Several of these compounds have been identified. However, identifying the unknown phenolic derivatives warrants further investigation.Future work is planned to identify these unknown peaks using the appropriate standards and by obtaining further mass spectrometric data. It would also be useful to compare compounds found after intake of PM with those found in the urine of participants who ingested white potatoes.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest. CT and EASAD were responsible for designing and conducting the research and preparing the manuscript. GM conducted analyzed the LC-MS analysis. NFS performed the plasma and urinary FRAP and total polyphenols analyses.

Address of institutions where the research was performed

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